



THE UNIVERSITY OF TEXAS AT AUSTIN
AUSTIN, TEXAS 78712

To J. W. Knowlton
✓ EAT *ed*
✓ WFS

Department of Zoology

October 4, 1979

Dr. E. A. Taylor
USDA/SEA
Area Director
Weslaco, Texas

Dear Dr. Taylor:

Enclosed are two manuscripts on techniques developed by Dr. Ellison and a copy of our quarterly report. If there are any questions, please let us know. Also, if you have any suggestions for improvement in form or substance, I would appreciate your letting me know.

Sincerely,

R. H. Richardson

R. H. Richardson
Professor

RECEIVED

OCT 15 1979

AREA OFFICE
WESLACO, TEXAS

First Quarterly Report
Cooperative Agreement #58-7830-9-120

October 1, 1979

1.0 SUMMARY OF RESEARCH

1.1

A total of 238 different sibships have been collected in the Arriaga area by SEA and U.T. Personnel. These collections include egg mass collections, larvae collected directly from the wounds, and collections of adult flies from traps and nets. These collections were frozen in liquid nitrogen and transported to Austin for laboratory analysis.

1.2

Analysis of 42 of these collections indicate that there are at least four different karyotypic strains which reside sympatrically in the Arriaga region. To date, no evidence has been found that indicates that these types interbreed in nature. Allozyme data from the same individuals that were used for karyotype analysis support both the classification of these collections into four different strains, and also the cytological evidence showing isolation of these populations.

1.3

An effort has been made (and is continuing) to develop reliable morphological methods for distinguishing different strains of screwworms by analysis of terminalia. Such methods would be applicable in the field without close support of laboratory facilities. Two distinctive types of terminalia have been documented from the West Coast of Mexico (both types are present at Hermosillo, Sonora), and these types are distinct from the terminalia of flies collected in Aldama (AC-17). The correlation of the terminalia type with the non-interbreeding strains is being tested by collecting in laboratory isolation single egg masses from wild-inseminated females captured in Arriaga. The eggs so obtained are then raised to larvae, sampled, and the remainder raised to adulthood. Thus from a single wild-inseminated female (iso-female line), both prepupa and adults are obtained. Data consisting of terminalia morphology and allozyme fingerprints are obtained from the adults, and karyotypic data and allozyme fingerprints are obtained from the larvae. Such collections are currently in progress and the female flies are being egged and raised at the Tuxtla plant. Confirmation by Dr. Ray Gagne of earlier results were likely thwarted by contamination of stocks kept at Mission (see 1.6 below).

1.4

The collections made by Commission and U.T. personnel during the spring and summer (before this cooperative agreement was effective) of the West Coast populations of screwworms were analysed and classified into four different groups:

NOTE: The * indicates those in culture in Tuxtla.

1. Those stocks like AEsD-1:

*AEsD-1	*M-19	*M-25
*M-28	*M-7	*NAVOLATO -1
SK-1	M-3	M-12
RPSS-1	RPSS-6	

2. Those like AC07

*AC07	*M-20	AC03
-------	-------	------

3. Those like GD1

*GD-1	*M-30	LF-1
CALS-1	CALS-2	CALS-3
RDT-3	RDT-4	

4. Those like M-4:

*M-4	*M-8	M-11
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1.5

The collections also during this time by SEA and UT staff from the Aldama region which were presumptive single egg masses or were from single wounds were classified according to their allozymes and karyotype. These are as follows:

STRAIN TYPES IN ALDAMA, TAMP.

Type 1	Type 2	Type 3	Type 4	Type 5
AC-7	AC-6	AC-4	C-1	HC-10
D-8	Canones	HC-8	M-14	
HC-9	HC-7			
HC-4	AC-1			
	CS-3			

1.6

Crosses among strains of the West Coast of Mexico, and crosses between strains of the West Coast and strains from Aldama were made by the SEA Research Group at Mission. These crosses were analysed at U.T. for karyotype, allozyme, and terminalia patterns. It was found that none of the crosses involving the West Coast strains could have come from the parents descended from the original stocks. Further investigations showed that the West Coast collections kept at the SEA facilities at Mission were heavily contaminated with strains of the Aldama type. The source of this contamination is unknown at this time. Additional strains from the Tuxtla facility have been requested. A full report on this problem will be jointly submitted by the SEA research group and the U.T. research group at an early date. It is interesting to note that initial observations of samples from these contaminated cultures have not revealed any hybrids.

1.7

A very interesting X-chromosome of reduced size was found by Dr. McInnis (SEA) in the heterozygous state in a collection from Aldama. On joint examination with Ellison (U.T.) it was determined that the decreased length was probably due to the absence of a secondary constriction (deletion of the Nucleolar Organizer Region is presumed), and certainly deserves further study. However, the autosomes and rest of the morphology of the X-chromosome conform to the "key" features used to identify one strain of screwworm, suggesting that the heterozygotes do NOT represent a hybridization between strains previously identified.

1.8

Behavioral studies are being carried out by SEA, APHIS and U.T. Personnel at the Tuxtla facility using screwworms derived from the Arriaga region. A large indoor observation cage has been designed and constructed at Tuxtla for observing mating behavior. In addition, field observations are being made during collection trips in the Arriaga region. Significant differences have been observed between screwworms in the Arriaga region compared to screwworms in the Northern parts of Mexico. First, the wounds do not have the distinctive odor so characteristic of screwworms elsewhere. Second, the adults do not seem to be attracted to the SWARM lure used in traps as readily as do adults from the border region. There were many instances where adult flies could be seen and collected from wounded animals and adjacent traps remained empty. Third, the infestations of animals, although numerous, do not seriously harm or kill as many animals as is characteristic of the screwworms in northern Mexico. Many ranchers do not treat their animals in regions where essentially every wound is infested. The losses to the ranchers are not as apparent as one would predict from experience with screwworm infestations in Texas and Mexico. The practice of some Arizona ranchers we noted in 1978 is more like that observed in the Arriaga region. Namely, some wounds did not receive treatment where it was difficult to administer, and the wounds healed in numerous examples.

2.0 UPDATED SPECIFIC RESEARCH OBJECTIVES

2.1

Of primary importance to both the Eradication Program and the genetic analysis of various strains is the determination of the sources of contamination in laboratory stocks, and the elimination of these sources. The presence of such contamination makes many of the necessary laboratory tests impossible, and clouds the interpretation of previous work where strain purity is an essential requirement. The U.T. group will work with the SEA personnel at Mission to solve this problem, doing that cytological, enzyme, and morphological analysis necessary to identify and isolate the presence and source of contamination in laboratory strains.

2.2

Simpler, and easily learned methods for the determination of screwworm type are badly needed. One major objective is to evaluate and document the terminalia morphology differences among Arriaga collections and test these results to determine if terminalia can be reliably used to indicate different isolated populations. It is anticipated that Dr. Ray Gagne will contribute greatly to this effort, once reliable material can be supplied to him. In addition to the standard microscope analysis of unstained and stained preparations, methods have been developed to employ the scanning electron microscope for these preparations. The use of this instrument should show much more detail of surface contours as well as offering better resolution and depth of field than conventional light microscope methods. Often features seen and documented using SEM can also be seen with light microscopy, once their presence and importance are known. The overall objective of this line of research is to make available a rapid method of screwworm identification that can be used in the field without extensive equipment or laboratory facilities, and with minimal training of personnel.

2.3

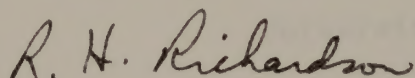
The analysis of collections from Arriaga will continue. This analysis includes the karyotypic analysis and documentation of at least 5 individuals from each collection, followed by the allozyme analysis of the same individuals. These data are to be analysed statistically using a cluster analysis program to be developed for analysing screwworm sibships. This will be an extension of the techniques developed for ticks (USDA cooperative agreement #58-7330-9-50). The completed programming for this computer analysis is expected next summer. Within 5-6 months portions of this analysis should be complete and some recommendations can be made concerning a test of the use of pure strains versus mongrel strains for the control of populations of mixed screwworm types.

2.4

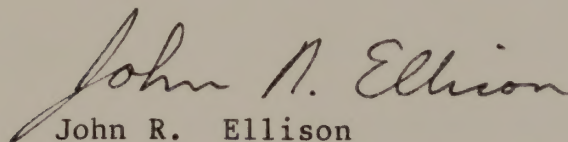
With the completion of the indoor environmental test room at Tuxtla, a series of behavioral experiments will be carried out. Initially the effort will be to induce "near normal" behavior, where the adults sit on leaves, visit wounded animals, feed and show courtship or copulatory behavior. These experiments will endeavor to document the mating behavior of the adult screwworm in an environment simulating natural conditions. Both mating behavior and mating preference experiments will be conducted in this facility after "near normal" behavior is achieved.

2.5

Field observations by SEA and U.T. personnel will continue in the Arriaga region. Special efforts will be made to determine where, and under the influence of what queues do the screwworm adults mate. This information will be then used in experiments conducted in the environmental room at Tuxtla.



R. H. Richardson



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AGE DETERMINATION IN ADULT MALE SCREWWORM FLIES (*Cochliomyia hominivorax*)

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AGE DETERMINATION IN ADULT MALE SCREWWORM FLIES (*Cochliomyia hominivorax*)

1.0 ABSTRACT:

The ascertainment of the age of captured adults is one very useful parameter in the characterization of wild populations of insects. This information has previously not been available. Described here is a method of determining the age of an adult fly in days, based on the number of concentric rings found on the ejaculatory apodeme. Each ring represents one diurnal cycle, thus one day of life. The test seems to be accurate at least up to 12 days when used with individuals in culture. The determination can be done using individuals that have been dried, frozen or preserved, and requires about 15 minutes.

2.0 INTRODUCTION:

Information concerning the longevity of individual members of wild screwworm populations can be used to establish an age profile of a wild population, and thereby correlated with other parameters of that population such as its propensity and ability to migrate, its ability to survive adverse weather conditions, and its inherent resistance to control by means of sterile male release. Several methods of age determination were attempted, including color determinations, testicular morphology, and the examination of various apodeme structures (patterned after several studies in Lepidoptera and in *Drosophila*). Of these, only the ejaculatory apodeme method gave consistent and precise results. This paper presents the

methodology for this determination.

3.0 MATERIALS AND METHODS

1. The flies are collected any one of several ways. Good results were obtained with frozen, fixed, and dried samples. In all cases the methodologies used are identical after the first step.
2. The entire posterior portion of the abdomen is excised and placed in 0.5N KOH, and heated to a temperature of 95°C. Depending on the method of preservation used, the time required in hot KOH varies between 5 and 15 minutes. Dried samples required more time, while frozen and fixed samples were generally free of extraneous material by 5 minutes.
3. The ejaculatory apodeme is a fan-shaped structure located between the hypandrium and the body wall. It is tenuously attached to the aedeagus by a small duct, and is easily separated from this structure. The ejaculatory apodeme is then dehydrated in 100% ethanol, cleared in xylene, and mounted in permount.
4. At a power of about 160X, concentric rings can be seen that correspond to days of apodeme growth. The innermost three rings are much wider than subsequent rings, and can usually be uniquely identified by their less pigmented color. Rings deposited after the third day are of rather uniform width and color. Observation with phase contrast helps little to distinguish the bands, but Nomarski interference optics greatly improves the visibility of the rings. All photomicrography was done with a Leitz Orthoplan

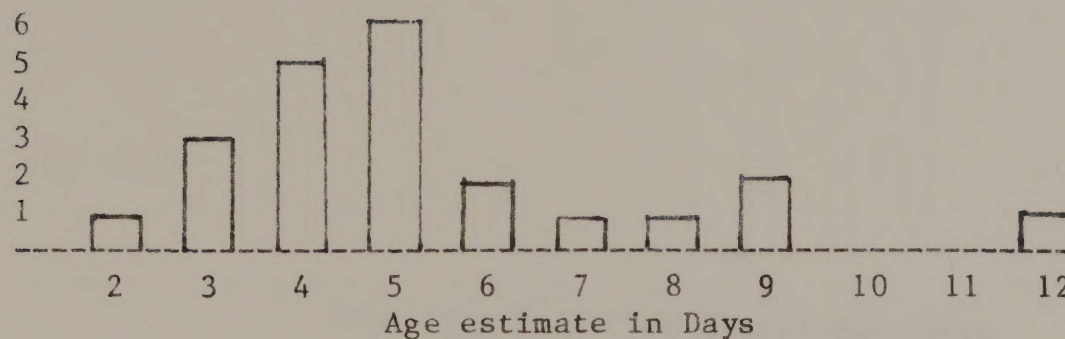
microscope equipped with Nomarski and phase contrast optics.

4.0 RESULTS

Figure 1 shows a series of ejaculatory apodemes from one to twelve days of age. Each has been marked to show the rings representing days of growth. Figure 2 shows an apodeme from a wild individual judged to be 8 days old when preserved. Since the structure of the bands from the wild individual are similar to those from laboratory reared individuals, it is proposed that determinations can be accurately made on wild individuals. It is impossible, however, to be sure that measurements on wild individuals correspond accurately to those made on cultured flies due to the difficulty of independently estimating the age of wild flies. In any case, the expectation that larger numbers of bands indicates greater age seem to be appropriate.

Using the cultured samples as reference, wild flies examined have had an estimated age of between two and twelve days, in a sample of 22 flies caught from the region near Arriaga, Chiapas, Mexico. These data are summarized in Figure 2. These results suggest that populations of screwworm have a mean age of just under 5.4 days. Since sexual maturity is achieved at 3 days of age, this suggests that the adult male has an average of only 2.4 days to reproduce during the summer months (July). These collections may be biased by the fact that the adults were collected in SWARM lure traps. Collections at other seasons of the year should yield data concerning average survival during the cooler fall and winter months.

FIGURE 3



5.0 ACKNOWLEDGEMENTS

The author is grateful for the support of the Texas Animal Health Research Fund, and The University of Texas for funds provided for the continuation of this project. The cooperation of the USDA/SEA personnel (especially Drs. Snow, Whitten, and McInnis) in providing materials for this study were also appreciated. The author is also grateful for the encouragement, and for the constructive review of this manuscript by Drs. Averhoff and Richardson of this department.

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Detecting Radiation Sterilized Males
in Screwworm Flies (Cochliomyia hominivorax)

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Detecting Radiation Sterilized Males
in Screwworm Flies (Cochliomyia hominivorax)

1.0 ABSTRACT

A rapid method of detecting radiation sterilized males in screwworm populations is presented. The method requires only 45 minutes as compared to earlier tests (LaChance and Rudd 1977) which required at least 4 hours (when modified from original procedure). The technique relies on cytologically detecting radiation damage to the chromosomes by the morphology of chromatin and nuclei during various stages of spermiogenesis. The technique is rapidly carried out, and requires only equipment that can be easily transported to the field. Since the determination of sterility relies on chromosomal breaks, losses and aberrations, this method should be readily adaptable to several species of insect pests.

2.0 INTRODUCTION

The effectiveness of released flies is determined through recapture, and subsequent calculation of fertile/sterile ratios. In carrying out this measurement, it is necessary to determine for each captured fly whether it is a factory reared, irradiated, sterile individual, or if it is a wild individual.

In the case of female flies, the determination is accomplished in the field. One simply dissects the female and observes the ovarian development looking for degeneration of the ovarioles. Previous methods for male flies, however, were not so easily adapted to small numbers in the field, (LaChance and Rudd, 1977, Reimann, 1967). The field methods of identifying "factory" versus wild males by visual examination of external morphology have proved neither accurate nor easily taught to novice collectors.

This paper presents methods for accurately determining if an individual has been irradiated. The methods can be carried out using equipment (dissecting and compound microscopes) that can be transported to remote locations. In addition, these procedures should be fairly general and adaptable to many species of insect pests other than screwworms.

3.0 MATERIALS AND METHODS

1. Testes are dissected in saline, and fixed immediately in modified Carnoy's fixative (Three parts absolute ethanol/one part glacial acetic acid) for 5 minutes. Methanol can be substituted for ethanol. A single testis is then placed on a slide in a drop of 45% acetic acid. When the tissue is soft (about 3 minutes) it is teased apart. A cover slip is then applied (22 X 30 mm.mm. work best) and the tissue is further dispersed by gently tapping the cover slip with a needle or with the tips of sharp forceps. A blotter paper is then applied over the cover slip, and the tissue is squashed with the thumb (hard) being careful not to move the cover slip.
2. The slide is then frozen in liquid nitrogen (within 10 minutes of squashing the tissue) and the cover slip is pried off with a razor blade. The slide is immediately placed in 95% ethanol and allowed to stay for about 5 minutes (or up to two weeks for storage).
3. The slide is removed from the ethanol and immersed for 15 minutes in a solution of RNase (Sigma #R5000, 20 micrograms per milliliter) at room temperature. The slide is then stained in Giemsa stain (4 milliliters of Fisher giemsa solution diluted to 40 ml. of water) for 8 minutes, then rinsed briefly in water, and immersed in T-butanol (Fisher Chemical Co.) with stirring for 2 minutes. The tissue is then mounted in Euparal, Diaphane, or it can be cleared in xylene and mounted in balsam or Permount.

4. Examination is best done with an objective of about 40X, making the total magnification for observation about 400X. Bright-field is usually sufficient for observation, but some slides may be more easily observed using phase contrast. A Zeiss Photomicroscope was used to photograph the figures presented.

If a phase contrast microscope is available it is possible to carry out this procedure without staining. The preparations are then observed immediately after step 1 in the above protocol. When this procedure is used, the total time for the procedure is reduced to 5 minutes. These preparations are not, however, permanent. They may be stained and made permanent after determination by continuing with the staining procedure.

There are two criteria for distinguishing irradiated from non-irradiated testes. First the condensed chromatid nuclei in irradiated specimens are irregular in shape and in size. There are large ones and extremely small ones. This is due to aberrant segregation of chromosomes due to breakage. Since aberrant segregation results in chromosome loss in some cells, and a net gain in chromosome number in others, some nuclei contain more DNA than others. The normal (non-irradiated) spermatid nuclei are round or heart-shaped and are of uniform size.

The second criteria (especially useful for younger specimens) utilizes the morphology of the chromosomes of a stage of meiosis

following pachytene when the chromosomes become more diffuse and have structures resembling lamp-brush loops. In non-irradiated squashes these chromosomes appear as long arrays of brush-like objects. In the specimens where the chromosomes have been multiply broken by irradiation, the nuclei at this stage look granular. No long arrays of brush-like objects can be seen.

4.0

RESULTS and DISCUSSION

The method presented here seems to be equally as accurate in the determination of fertility as the method of LaChance and Rudd, 1977, and can easily be carried out in the field. The method relies on detecting radiation damage to chromosomes which results in either breakage of chromosomes or chromosome loss during divisions. The results of chromosome damage by radiation treatment are particularly evident at two stages of spermiogenesis. At a stage after pachytene (but before diplotene) the chromosomes become somewhat diffuse and are visible as lampbrush-like structures. In the normal, non-irradiated individuals, these chromosomes can be seen as long arrays of wispy, beaded structures (See Figure 1). Each cell has six such structures. In the irradiated samples (given approximately 6800 RADs) such long structures are broken into many fragments. The nucleus appears granular and lacks long arrays.

The second criterion for identifying irradiated individuals depends on the segregation of genetic material at the meiotic divisions. Irradiation at the level given for sterility induces a large number of chromosome breaks (8 to 10 per cell). These breaks heal to a certain extent and form chromosome elements with translocations, dicentric chromosomes, and inversions. The net result is that segregation of genetic material during subsequent divisions is quite erratic, and this frequently results in spermatid nuclei with large variations in their DNA content. In addition to the criterion of size differences, the spermatid nuclei often have ragged edges

(probably due to the formation of chromosome bridges at anaphase). Normal spermatid nuclei, in contrast, are of generally uniform size and have a smooth profile (see Figure 2).

The methods presented here are relatively independent of the age of the individual. Males were examined at several intervals between one and twelve days of age. In individuals of all ages, adequate cells at the lampbrush stage and adequate spermatid nuclei could be found for proper classification. In older irradiated males, however, spermatid nuclei were relatively rare even though non irradiated individuals of the same age had many spermatid nuclei.

Using two independent criteria for such a determination, multiplicatively increases the sensitivity and the reliability of the method. A method similar to this one should be effective in almost any species of insect that has centromeric chromosomes. The methods probably would be of less use among those species of insects with diffuse centromeres.

5.0 ACKNOWLEDGEMENTS:

The author wishes to thank the Southwest Animal Health Research Foundation, The Perry Foundation, and the administration of the University of Texas at Austin for generously providing the funds for this work. The author is indebted to Drs. Broce, Whitten, and McInnis (USDA/SEA at Mission, Texas) for providing frozen material, information, and encouragement for this work. The author is also deeply grateful for the encouragement and enlightened comments by Drs. W. A. Averhoff and R. H. Richardson, University of Texas at

Austin.



THE UNIVERSITY OF TEXAS AT AUSTIN

AUSTIN, TEXAS 78712

Department of Zoology

October 10, 1979

Dr. Ray Gagne
USDA/SEA/AR
Beltsville Agricultural Research Center
Beltsville, Md. 20705

Dear Ray:

I just received from Dr. Snow a day or two ago a copy of your report of September 18 to Dr. Lindquist. Of course, you had discussed these interpretations with me earlier, so I was not surprised with that, but I am disappointed that we didn't get the chance to go over together the preparations we have made. I don't think that all the things we are impressed with were included in your evaluation. Also, you may have received a copy, but just in case you didn't, I'll send you our quarterly report telling of the problems of contamination in the stocks at Mission. We have not had a chance to evaluate the situation for Tuxtla. As John and I mentioned to you during our brief visit at the airport in McAllen some time ago, such problems would seriously interfere with your evaluation.

I hope you will consider the confusion unreliable material could cause, and will reconsider what I take to be your present position, namely, that there is not much in the screwworm types we have identified to demand further attention from you. We feel very strongly that your help is needed, and I hope you will continue actively working on these types using new specimens.

In your report there are several points we could discuss, but I think they largely are irrelevant to the crux of the things we have found so far that apply directly to the screwworm eradication program using sterile male release tactics. Many definitions of species, of course, are used in different contexts, and the semantic problems are gargantuan. Nevertheless, there is very strong evidence indicating little or no hybridization between several of these types under natural conditions. Whether one labels them different species, or, as we prefer, gamodemes, is tangential to the eradication efforts. We need morphological features to more easily separate members of two sympatric non-interbreeding populations in order to better evaluate their significance to the program, under un-natural conditions of sterile fly release. Your experience and access to notes and collections place you in a unique position to contribute to this objective.

If it is at all possible, I would like for you to spend some time in Austin, where we could compare notes and interpretations, and come to some agreement on the significance of the differences we have seen in the terminalia. Wil Averhoff will have new material taken from offspring grown from wild females that have been individually egged in Tuxtla,

thereby minimizing the chances of contamination. Although these are collections from Arriaga, they likely will include some of the types similar to those we have seen both in Aldama and in western Mexico. By the time you would arrive, we would have the chromosome and protein data to categorize them according to reproductively isolated populations.

If you are able to attend the next technical meeting in Mission, as Dr. Snow suggested, this would be an ideal time to include in your itinerary a stop-over in Austin. Although John and I are tied down with a teaching schedule, we would have plenty of time to spend on the problem with you. The best time might be the weekend before the technical meeting, if you could fly in on Friday night or Saturday morning before the meeting, which usually is on Tuesday.

Sincerely,

R. H. Richardson
Professor

cc: J. W. Snow
B. Hightower
R. W. Hodges
L. V. Knutson
L. LaChance
D. A. Lindquist
M. E. Meadows
N. L. Meyer
B. Miller
Mission & Tuxtla Researchers
J. Novy
N. V. Pineda
E. A. Taylor
M. Vargas
L. E. Wendel
D. D. Wilson

Dynamics and Stability

of

Phyletically Packed Communities

September 12, 1979

Renewal Proposal, DOE Contract

DE-AC05-79ET01834

Departments of Zoology and Physics

University of Texas at Austin

R. H. Richardson and Jack S. Turner

with assistance of

Dan Vasco

2.1.2 Identification Of Gamodemes In Populations -

The analysis of population structure has been hampered by the absence of tools to identify breeding units. Unless the members of populations were identifiable by striking visible or similar appearances, a subdivided local population would remain completely undetected. Workers generally were forced to assume homogeneity in natural populations even though detailed studies invariably indicated the presence of heterogeneity.

We have almost completed the reprogramming of analyses developed by Makela (1975) and Makela and Richardson (1977) to make them more easily used, better documented and probably more reliable. Since the data input format differs from earlier versions, we have delayed the completion of the analyses of the last year study in Hawaii until now. In addition, Dr. Peter Smouse, University of Michigan, and I have been developing a multivariate analysis of mixed gamodemes, which will be very useful in the complete analysis of such data (see Appendix C for draft of the manuscript). Therefore, the analysis of Hawaiian Drosophila, particularly D. mimica, is reaching a point where the results soon will be summarized for publication.

The results for 1974 and 1976 indicate that at least two gamodemes are identifiable in Bird Park, and their proportions change. In an earlier study a single homozygote of APH declined during a period of very acid rainfall during a volcanic eruption, but the original proportions of "genotypes" in the population returned quickly (Rockwood, 1969). We earlier postulated that the recovery might have reflected migration into the affected population from a population in a protected area receiving less rainfall (Richardson and Johnston, 1975), rather than selection against this particular electromorph. However, the most probable explanation is neither selection on this particular locus nor migration, but that the locus is a marker which distinguishes between two gamodemes. One gamodeme may have been more severely depressed by the adverse conditions during volcanic eruption, but recovered rapidly after the eruption ceased, or, alternatively, the fluctuation represented normal seasonal fluctuations of separate populations. Consistent with this latter interpretation is the recently published result of similar allozymic fluctuations in the absence of an eruption (Steiner, 1979).

2.2 Screwworms

The idea of eradication of a species by the release of large numbers of males sterilized by massive doses of X-rays was conceived about 25 years ago by Dr. E. F. Knipping and developed by him, Dr. R. C. Bushland, and their colleagues. The screwworm has been successfully eliminated from several Caribbean islands and from the southeastern United States. A "barrier zone" along the U.S.-Mexico border was established in 1964, with the intent of preventing a re-invasion of the United States each spring by the populations surviving below the freeze line. This barrier zone was effective for several years, but effectiveness in the last decade or so has been erratic.

In a recent symposium (Richardson 1978) we sketched a number of areas in which genetic and ecological processes could be factors contributing to the difficulties experienced by the "eradication" program. From our perspective outside the USDA program, it appeared that this massive effort in pest control had initiated a very interesting experiment from which we could learn many features about the responses of a community placed under intense stress. Our suspicions were that the pest was comprised of one or a few component species that would be differentially stressed, depending on their relationship to the population being released (Makela and Richardson, 1979). Indeed, we have discovered that this suspicion was substantially correct.

The results of the genetic analyses during the past year may be summarized briefly. There are many cryptic gamodemes (and presumably are different species or semi-species), many of which are sympatric. We have identified at least 7 such groups in the United States last year, 3 of which were found below the freeze line last winter in Mexico, and the others are yet to be found again this year. All of these were collected while up to 3000 sterile flies were being released per square mile per week, and control was limited to population suppression rather than eradication. The released flies were of a different type than any of those identified, however. While some of the types have been found only above the freeze line, their method of surviving freezing temperatures is unknown. Diapause or other adaptive responses not previously associated with the genus may be involved. We have found several additional species in Mexico which have not been found in the United States.

The genetic techniques used to distinguish the cryptic species involve chromosome karyotypes derived from quinacrine-stained metaphase chromosomes (developed and performed by Dr. John Ellison) followed by electrophoretic analyses of the same larvae from which the chromosome preparations are prepared. The collections of larvae typically are obtained from naturally infested wounds in livestock (collected by or under the guidance of Dr. W. W. Averhoff). The karyotypes occur in distinct groups, differing by heterochromatin concentrations (primarily on sex chromosomes) and chromosome morphology. We have not found types precisely the same as those with karyotypes published before the construction of the barrier zone. Thus, it appears that either these types have, indeed, been eradicated and now are extinct, or else they have been suppressed and/or their geographic range has been reduced such that our sampling has failed to reveal their presence. Concordant with the chromosome groups are sets of some allozyme electromorphs unique to some karyotypic groups, while there are strong differences in frequencies in other cases. In one instance a pair of sympatric groups are distinguishable only by allozyme assay.

Natural heterozygotes have not been found, suggesting natural hybrids are rare or non-existent. We have examined samples from more than 200 wounds, some of which represent a few matings. Egg clutches of different females may occur in a single wound. These cases are often observed when there are larvae of different instars of development. Many of these taxa are sympatric, and collections may be made of several types in the same area, sometimes from the same wound.

We have found examples suggesting strong differences in host preferences, even between such similar hosts as cattle and sheep. Niche separation in this way closely parallels the plant substrate preferences in Drosophila we have studied previously. Also similar to Drosophila we have studied, there is accumulating evidence that some species oviposit less than a dozen eggs in a clutch in some individual hosts, while others are more likely to deposit up to about 50 eggs, and a few typically deposit up to 200 eggs. In the latter case and in some of the intermediate cases they also will re-oviposit on previously infested wounds, whereas in some cases there is only one oviposition per wound. Clearly these species represent a wide range of reproductive strategies, from "r-selected" to "K-selected" species.

As will be addressed in the section below, this extreme diversity of reproductive strategies between closely related, sympatric species is intriguing. As in Drosophila, such diversity of screwworms is very difficult to discover by casual observation of individuals. In Drosophila we commonly discover the existence of some of the cryptic species when we attempt to culture certain types. Since the K-selected species often have specialized cues for oviposition, and often lay only a few eggs, culturing in the laboratory is a challenge. When we have a "single species" which is easily cultured, but then find certain lines are very difficult to culture, we have learned to take this anomaly as a strong clue of the presence of a cryptic K-selected sibling species. Such anomalies will likely be important in future studies, whether they involve Drosophila, screwworms, or other taxa.

The geographic ranges of the cryptic species of screwworms also are highly variable. Some extend over a thousand miles. One type is found from Mazatlan to Phoenix, while another sometimes extends between San Antonio and El Paso and south from San Angelo across "west Texas," presumably into the Chihuahuan Desert. Still others have only been found in a single locality, such as north of Tampico within 175 miles of the Gulf of Mexico and with a north-south range of about 200 miles. A few have only been found in a single collection, which may suggest either a restricted range, that or they occur in very low frequencies, causing difficulty in sampling.

Although only historical speculations may be made at the present time, it is plausible that the initial successes of the eradication program represented the extreme suppression, or possible extinction, of a dominant species in Florida and Texas, with subsequent outbreaks representing the increase of previously secondary species. As the sterile males to be released in the future are matched to specific species, we will be able to observe changes in the relative proportions of the remaining species. Thereby "species releases" (significant and permanent expansion in numbers or the range of a natural population) for secondary species will be monitored. Also, it is likely that we will relate some of these census and geographic changes of the species to weather and other variations occurring over a few years. These factors may demonstrate some characteristics besides competition or interference by related species which have restricted the species ranges or retarded their seasonal increase.

Although this contract provided part of the support for this work last year, this will not be the case in the renewal period. We presently are continuing some of the genetic and systematic surveys associated with pest control with the financial support of the USDA and the livestock industry. Although this work is focused on the control or eradication of a pest (which we found to be a complex of species), we will continue to accumulate data of more basic interest.

With the theoretical studies proposed for this contract in the next period, we hope to gain some insight into the community dynamics ("species release" of secondary species, as various numerically dominant ones are eliminated or suppressed, for example) and develop appropriate models for the type of responses precipitated by an autocidal biological control program or any other selective reduction in the Malthusian parameter for the intrinsic rate of population increase. In addition, responses of other members of a community and the resulting effects on stability of the community will be brought into focus by the models.

2.3 Ticks

The research on ticks has been supported by the USDA during the past year (while this Contract with DOE has supported the research on Drosophila and screwworms). However, for completeness of the unpublished background data as it relates to our plans for the next contract period with DOE, a brief review of this work will be given.

Ticks represent a different type of experimental material to either screwworms or Hawaiian Drosophila, having received a concerted effort over the past several decades to reduce the population by chemical insecticides. In some cases, eradication of particular species by insecticide treatments over large areas has been attempted. (They are vectors for a number of diseases of livestock and humans.) Our work has centered on those which have received persistent treatments of various insecticides for a number of years. In certain cases species have managed to extend their ranges greatly in the face of such stresses as the insecticide treatments. Clearly the community has been altered, likely by human activities, but not necessarily to the detriment of the pest species. We are now interested in identifying the reproductively isolated populations (gamodemes, which in many instances probably represent cryptic species). From this base line we hope to gain some insights relating the range extensions to the factors responsible for range extensions, whether they be reflections of genetic changes in the population, or other factors having a closer connection to environmental modifications by man. The understanding of community instability, whether reflecting changes involving local or absolute extinction, or greater packing of a local community by the range extension into a new area, represents the broad objective of this research.

In collaboration with USDA researchers (coordinated by LaWanda Hunt) we have identified several cases of presumptive cryptic species in three presently recognized species of ticks. We are now in the process of identifying the ranges of each gamodeme, as well as conducting various breeding tests among the several populations we have found.

GENETIC DIFFERENCES AMONG SCREWORM POPULATIONS

ANNUAL REPORT, USDA Agreement No. 58-7B30-9-120

25-Aug-80

R. H. Richardson, J. R. Ellison & W. W. Averhoff

UNIVERSITY OF TEXAS AT AUSTIN

O/H Graham

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ANNUAL REPORT, 1979-80

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1.0 CHROMOSOME ANALYSIS OF SCREWORM POPULATIONS

1.1 Statistical Analysis

The more detailed analysis of chromosomes from collections in Chiapas is underway, and the statistical analysis of all collections made during the past 3 years is proceeding. Computer programs for entering the data in appropriately formatted files and for a nested ANOVA (strains, individuals/strains, and cells/individuals/strains) has been written. This program has the option of producing a plotter output of the karyotype including the standard errors.

The lines selected to be included in a "standard" file for each gamodeme were made with the following criteria: (1) no apparent genetic oddities from the majority of the strains identified subjectively as members of the particular gamodeme (e.g. stocks derived from crosses), (2) addition of the strain to the set did not cause a significant F-value for arm length, total length or arm ratio, (3) the line was not contaminated with individuals of another gamodeme at the time samples were taken for analysis. In addition, attempts were made to include strains from a wide geographic region and across all sample times. Where there was a known or possible polymorphism in the Y-chromosome, the strains were merged into one gamodeme, provided no other chromosomes resulted in violation of conditions above. In this way we have attempted to reduce the number of distinct karyotypes to a minimum. We have labeled the resulting 10 gamodemes A through J (Table 1).

The analysis of each strain requires measurements of all the chromosomes in a cell with a complete analyzable set of chromosomes. The measurements are made from photographic enlargements, which are expensive and time consuming. To our knowledge, there has not been a project similarly detailed in karyotyping unless at least partially automated (e.g. human karyotyping). While the identification is much easier and faster, the quantification of the karyotype is very tedious. After the measurements have been made, they are entered into the data files with the program, DATFIL, written to be used in combination with the editor, TECO. This combination allows data manipulation (updating, correcting, sorting) to be very flexible.

TABLE 1.
Strain Membership in Gamodemes (Analysis Completed)

Latest Revision: 8/15/80

1. Gamodeme A --		
AEdD-1	M-7	SK-1
M-12	RPSS-6	RPSS-1
GD-1	CALS-1	CALS-2
WA-3	RDT-3	RDT-4
LF-1		
2. Gamodeme B --		
ACO-3	ACO-7	
3. Gamodeme C --		
M-20	RR-26	RR-55
4. Gamodeme D --		
M-25	RR-1	IMR-14
IMR-8	HLR-64	
5. Gamodeme E --		
RR-11	RR-22	IMR-10
RPSS-2	RPSS-4	RDT-7
6. Gamodeme F --		
RR-23	RR-24	RR28
HLR-42	VC-4	VCI-F3
JAC-12		
7. Gamodeme G --		
AC-4	HC-8	
8. Gamodeme H --		
M-14	C-1	CS-13
LLS-1	CS-9	ALD-2
AAP-1		
9. Gamodeme I --		
D-8	HC-4	AC-7
HC-9	AC-1	AC-6
Canones	CS-3	
10. Gamodeme J --		
BE		

1.2 Nomenclature

It is important that data subjected to karyotypic analysis be taken from fully analyzable cells, otherwise normalization of the lengths is not possible. Use of non-normalized data results in measurements that cannot be compared, cell to cell, individual to individual or strain to strain. While arm ratios will be constant for raw or normalized data, individual arm measurements are useless. The criteria for identifying each chromosome is as follows:

1. Sex chromosomes -- obviously the X-chromosome is common to males and females, and the Y-chromosome is the one found in males alone as a single item. Usually the Y-chromosome is more heterochromatic, thereby appearing brighter in fluorescent microscopy using quinacrine stain. These are the smallest chromosomes.
2. Chromosome 2 -- the longest of the autosomes, and has small bright regions of centromeric heterochromatin on both sides of the centromere.
3. Chromosome 3 -- the second longest chromosome, and has large bright centromeric heterochromatin, usually symmetrically distributed on either side of the centromere.
4. Chromosome 4 -- the most nearly metacentric of the remaining autosomes, without Q-bright centromeric heterochromatin
5. Chromosome 5 -- the most nearly submetacentric of the remaining autosomes, without Q-bright centromeric heterochromatin
6. Chromosome 6 -- the most nearly acrocentric of the autosomes.

Total length and centromere position vary distinctively among most gamodemes in at least one chromosome, or in several chromosomes to lesser degrees for the more similar types (Tables 2 and 3).

A number of polymorphic features have been noted in passing, and some have been identified as polymorphisms after McInnis (SEA) noted differences between homologous chromosomes in a single cell. These include a nucleolar organizer mutant and variations in interstitial Q-bands. These are indicated on the karyotype with a +/- designation. These details are not complete, but are added as they are noticed.

1.3 Karyotypic Patterns

When the karyotypes are compared, ignoring the Y-chromosomes, there are two groups of similar types. Gamodemes E, F and I form one cluster, and Gamodemes A and H form another cluster. These two clusters, in turn differ from one another primarily in the length of the long arm of the X-chromosome, the E-F-I cluster having a longer arm than the A-H cluster. Within these clusters only E and F are known to occur together

TABLE 2.
Chromosome Metrics for Gamodemes -- Total Length

Gamodeme	Chromosome						
	X	Y	2	3	4	5	6
Type A	.034 .033-.035	.024 .022-.026	.111 .109-.113	.097 .096-.098	.083 .082-.085	.090 .089-.092	.082 .080-.084
Type B	.043	.025	.109	.105	.089	.081	.074
Type C	.036	.023	.113	.093	.092	.085	.077
Type D	.036	.023	.112	.093	.089	.082	.083
Type E	.038 .035-.040	.026 -----	.109 .106-.111	.093 .090-.095	.091 .089-.092	.086 .085-.087	.082 .081-.084
Type F	.037 .035-.039	.029 -----	.111 .109-.113	.091 .089-.925	.085 .082-.087	.086 .083-.088	.087 .085-.089
Type G	.039	<u>.036</u>	.114	.087	.077	.078	.094
Type H	.035	<u>.019</u>	.112	.096	.083	.090	.082
Type I	.041	.023	.110	.097	.083	.082	.083
Type J	.037 .036-.038	---- -----	.115 .114-.116	.092 .090-.094	.086 .083-.089	.083 .082-.084	.086 .084-.088

TABLE 3.
Chromosome Metrics for Gamodemes -- S/L Arm Ratio

Gamodeme	Chromosome						
	X	Y	2	3	4	5	6
Type A	.271 .259-.283	.017 .000-.035	.719 .715-.722	.752 .741-.762	.775 .743-.806	.613 .598-.629	.343 .330-.356
Type B	.320	.000	.819	.679	.719	.547	.518
Type C	.268	.000	.666	.594	.674	.602	.525
Type D	.234	.564	.721	.800	.583	.580	.401
Type E	.254 .238-.271	.000 -----	.675 .658-.692	.739 .714-.765	.757 .752-.790	.653 .628-.678	.310 .293-.327
Type F	.232 .217-.246	.362 -----	.721 .693-.748	.791 .770-.811	.736 .708-.765	.661 .620-.703	.351 .333-.369
Type G	.280	.643	.672	.706	.647	.610	.454
Type H	.307	.162	.719	.793	.772	.630	.324
Type I	.207	.483	.707	.819	.756	.643	.357
Type J	.275 .251-.298	---- -----	.727 .694-.759	.763 .725-.800	.742 .700-.785	.643 .609-.677	.403 .387-.418

Gamodemes B, C, D and G are vastly different from the two clusters, and from each other. Nevertheless, C is more similar to the E-F-I cluster and D is closer to the A-H cluster than are the other two (B, G). Gamodeme G is moderately similar to the A-H cluster, but differs considerably from D. Gamodeme B is more similar to the E-F-I cluster, but differs much more than does C (Figures 1 and 2).

B-----C-----~~(E-F-I)~~-----A-H-----D-----G

Long X Short X

1.4 Possible Phylogenetic Relationships

The group, E-F-I, may be a polytypic species, or maybe the gamodemes represent a sibling species complex. This cluster may be taken as primitive. Then a sequence of species could have formed along the west coast, (E-F-I)-->C-->B. The same type of process, occurring simultaneously on both coasts would have given rise to A in the west, and H in the east. G could have formed from D during the last Ice Age, when all the populations were pushed together in the south, and then G moved to the northeast and D moved to the northwest as the Ice Age ended and warm-adapted organisms moved northward.

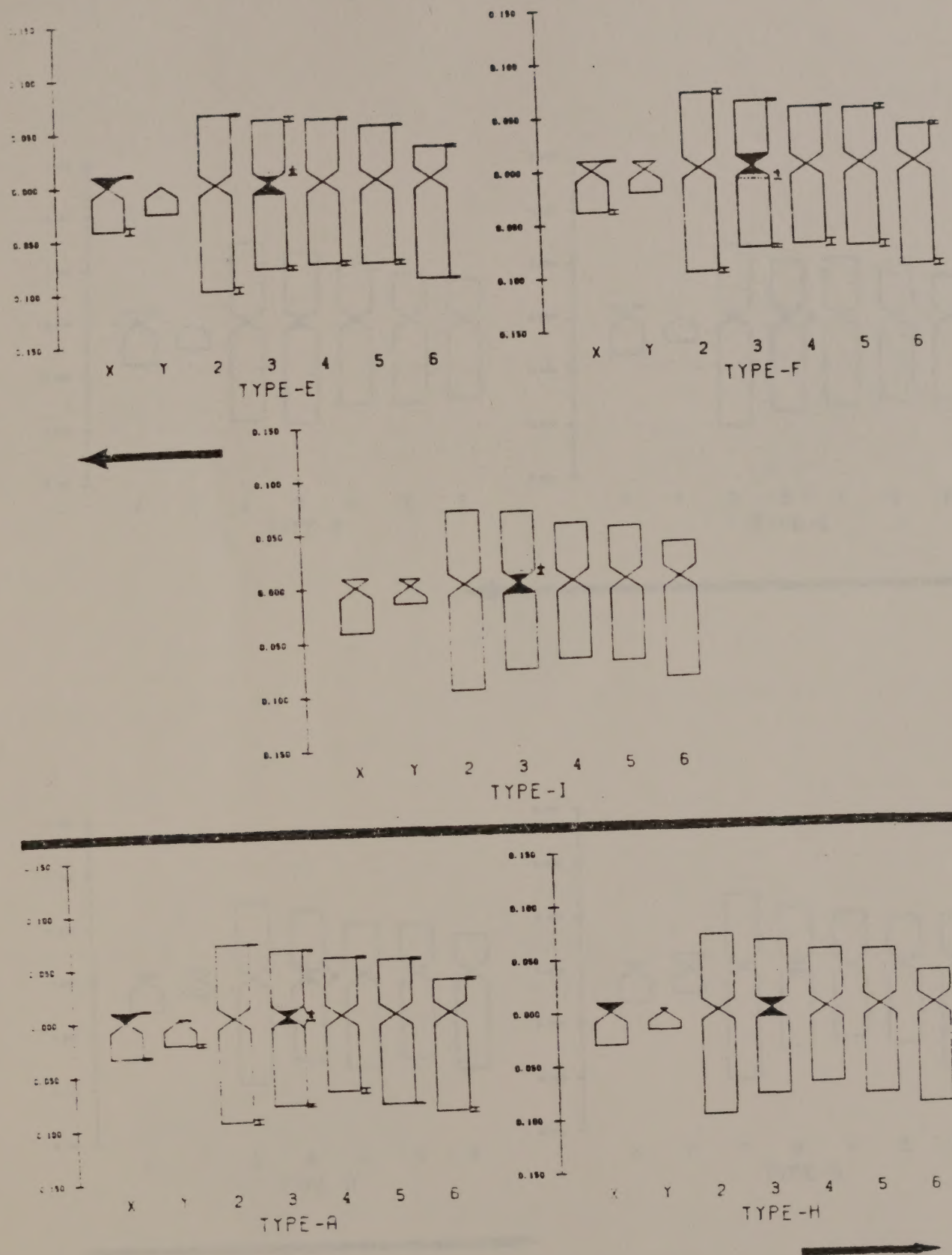


Figure 1. Computer plotted karyotypes of the gamodemes showing their relationships. The Y axis indicates the proportion of the total genome. Darkened regions are quinacrine bright regions characteristic of the gamodeme. Variable regions of quinacrine fluorescence are indicated by dotted lines.

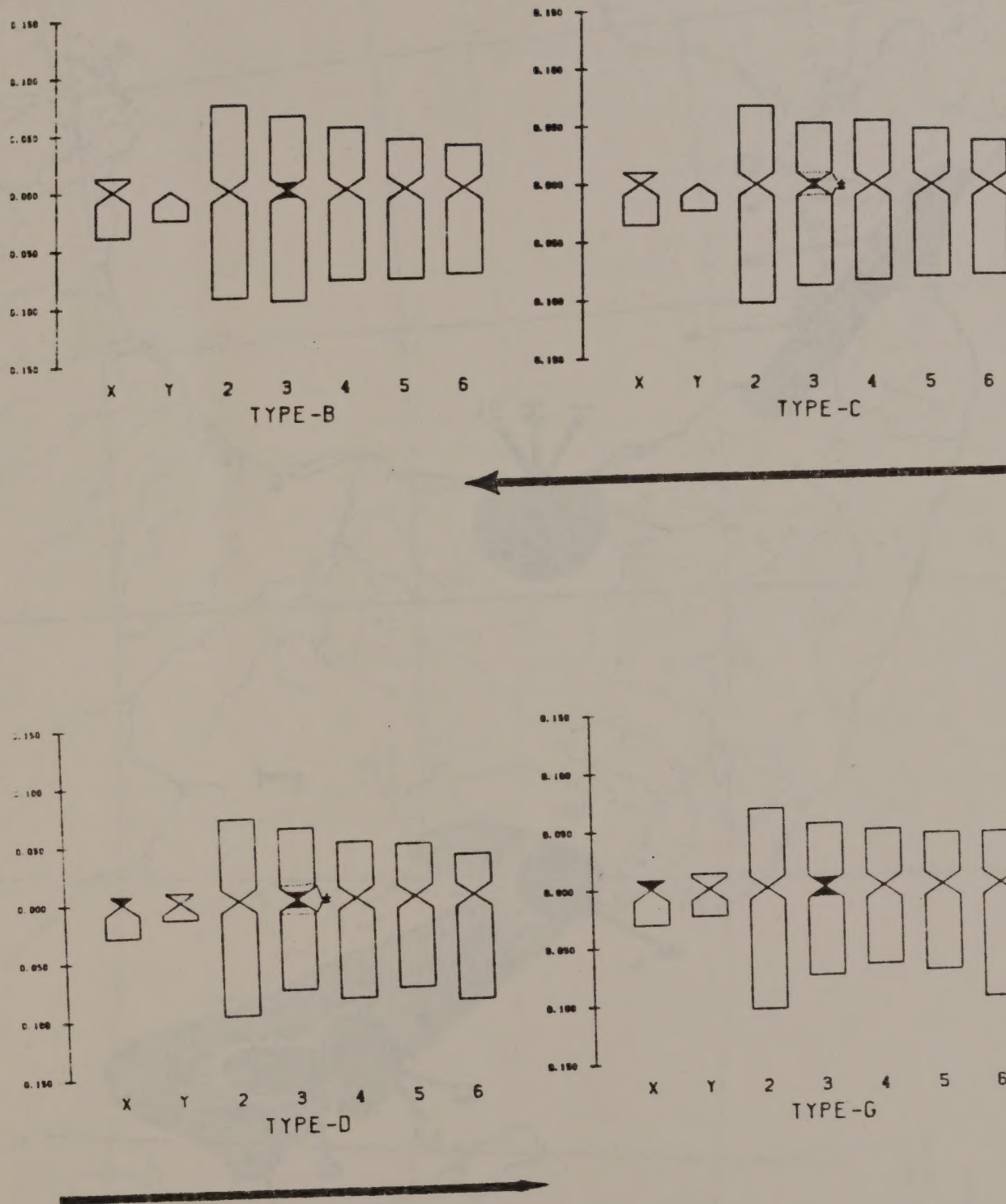
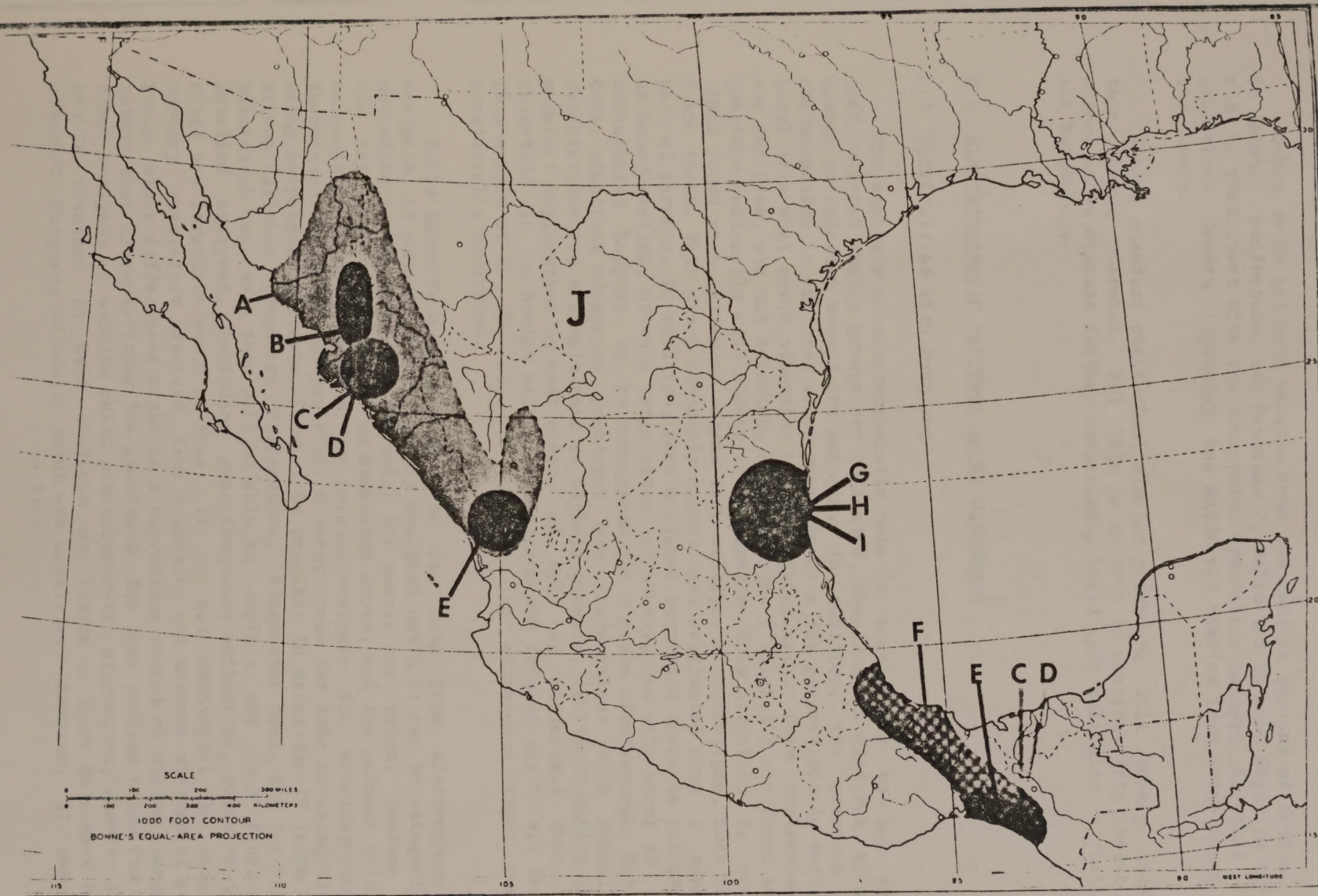


Figure 2. Computer plotted karyotypes of the gamodemes. The karyotypes are arranged in the order of their similarities as indicated by the arrows on figures 1 and 2. For a discussion of this relationship, see part 1.3 above.



Since we do not have samples from the central regions of Mexico, it is mostly conjecture, but Gamodeme J, collected in West Texas in 1978, also is consistent with the *Drosophila* speciation pattern, in that the Chihuahuan Desert typically has different species from those found on the coasts.

A more standard statistical cluster procedure will surely modify this scheme somewhat, but this is helpful to consider this tentative scheme as we discuss further comparisons based upon molecular variation and morphology.

2.0 ELECTROPHORETIC PATTERNS AMONG GAMODEMES

2.1 Difficulties With Sampling

The analysis of electrophoretic data is complicated by the fact that full-sib family groups are collected, sometimes in mixtures, if egg masses or infested wounds are sampled. In addition, the presence of contamination in cultures after only one or a few generations in culture complicate what would otherwise be unambiguous data. When contaminants are included, and not detected, electromorphs ("alleles") not found in a gamodeme ordinarily will appear in the sample. This situation is sometimes found when, given an enzyme with multiple electromorphs, more than 5 are found. This group obviously cannot have originated from a single pair of parents. In addition, when mixtures occur and there is little or no interbreeding, often a large excess of homozygotes will occur if the contaminant happens to be homozygous for a given electromorph. For F_1 data, sometimes offspring genotypes are inconsistent with Mendelian expectations for a single pair of diploid parents. (Multiple mating is a possible explanation in most cases, as well as mixtures.) When the objective is to assign values of electromorph frequencies to all forms in a sample of 0, .25, .5, .75 or 1., the extraneous types cause considerable confusion.

While there is a large backlog of raw data from electrophoretic analysis, it cannot be meaningfully analyzed until it can be categorized according to the gamodeme from which the sample was taken. Because of the internal correlations as described above, and the unknown allelic composition of the gamodemes, the initial analyses via electrophoretic techniques is much less powerful. However, once the data is categorized using karyotypic criteria, it will be possible to determine if there are strong allelic frequency differences, diagnostic loci, and the like. Subsequent data sets then will be able to exploit any such findings. Detailed analyses, including gene frequency estimation, prior to this stage are largely a waste of time. It is not meaningful to examine summary statistics derived from a complex and unknown mixture of gene pools, since differences might be a capricious result of mixing samples rather than characterizing the gamodemes. The same problem holds for any other analyses or studies using electrophoretic approaches, whether from adults obtained at bait stations or larvae as these data have been obtained. Mixtures of adults which are unrelated, however, if sampled

adequately, may be analyzed with the computer programs we have developed for *Drosophila* and ticks, thereby possibly identifying independently the gamodemes, or possibly further subdividing gamodemes which are not resolved with present cytological techniques.

2.2 Difficulties With Inheritance

There remain several enzyme assays which badly need genetic data for aid in the interpretation of the electrophoretic patterns. In an earlier report and in discussions with Jim Whitten (SEA) it is clear that PGM produces a complex electrophoretic pattern needing crosses to unequivocally score the homozygotes and heterozygotes. Also, HBDH seems to have only single bands, but it is more likely that some of them are multiple, more diffuse, and stain more lightly. If it was known that this type of band were actually a heterozygote, then accurate scoring of the patterns would be possible. In many instances the old assays may be rescored from photographs when the genetic tests are completed, giving a significant amount of additional information. These tests are badly needed, but must be conducted in security.

2.3 Preliminary Summary

It is premature to summarize the frequencies of the various allozymes, but a few aspects are fairly clear at this time. The loci which seem to be the most interesting are GOT, MPI, EST-D and PGM. Some statistically significant differences are found between the populations on west coast compared to those on the east coast, but as discussed above, this is not very meaningful considering the diversity of populations on either coast.

3.0 COMPARATIVE MORPHOLOGICAL STUDIES OF MALE GENITALIA

3.1 Comparative Anatomy

Methods were developed for studying the genitalia using both the scanning electron and the light microscope. For light microscope examination the methods usually used in *Drosophila* worked well with some modification. The intense pigment present in screwworm genitalia hid some of the details and for this reason methods were developed to remove much of the pigment. The male genitalia were then stained in Cason's stain (a modification of the standard Mallory trichrome stain) in order to differentiate the various parts. This method allows the observation of all of the characteristics described from scanning EM observations (Figures 4, 5, 6).

The literature offered scant information as to the optimum methodology for preparing genitalia for scanning EM. The method used here is simple, and can be carried out using genitalia that have been previously

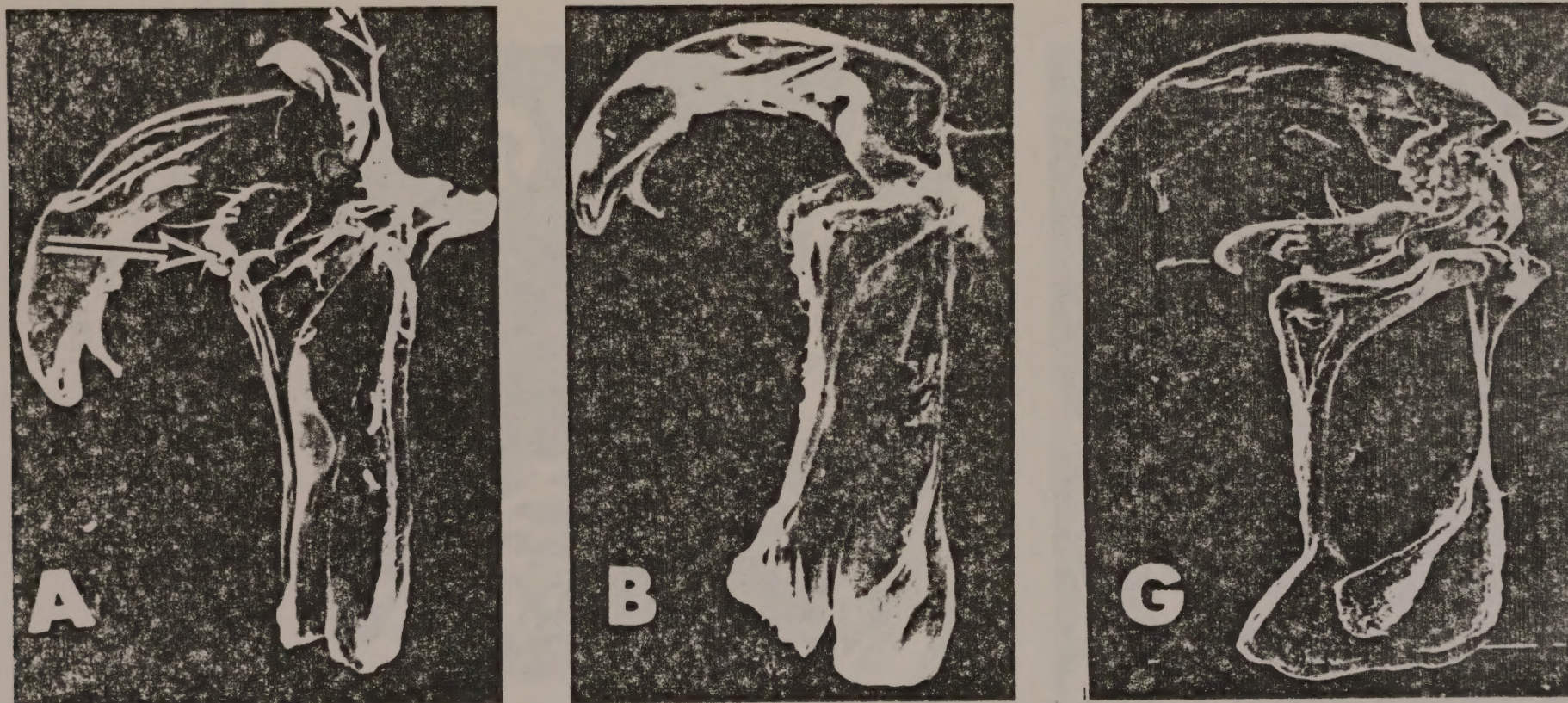


FIGURE 4: These lateral views of the genitalia of three different types are reproduced at a total magnification of 90X. The top of the figure is the posterior of the insect, the dorsal side is to the right. On figure A, the epithalis, gonopod, and parameres are indicated with a short arrow, a long arrow and an open arrow respectively. Note the difference in size between type A parameres and those of type B (the two figures are at the same magnification. The paramere of type G is laying across the gonopods (and thus extending ventrally rather than the posterior orientation of types A and B). The epithalis of A projects posteriorly (toward the top of the figure) while the epithalis of B and G project dorsally (toward the right).

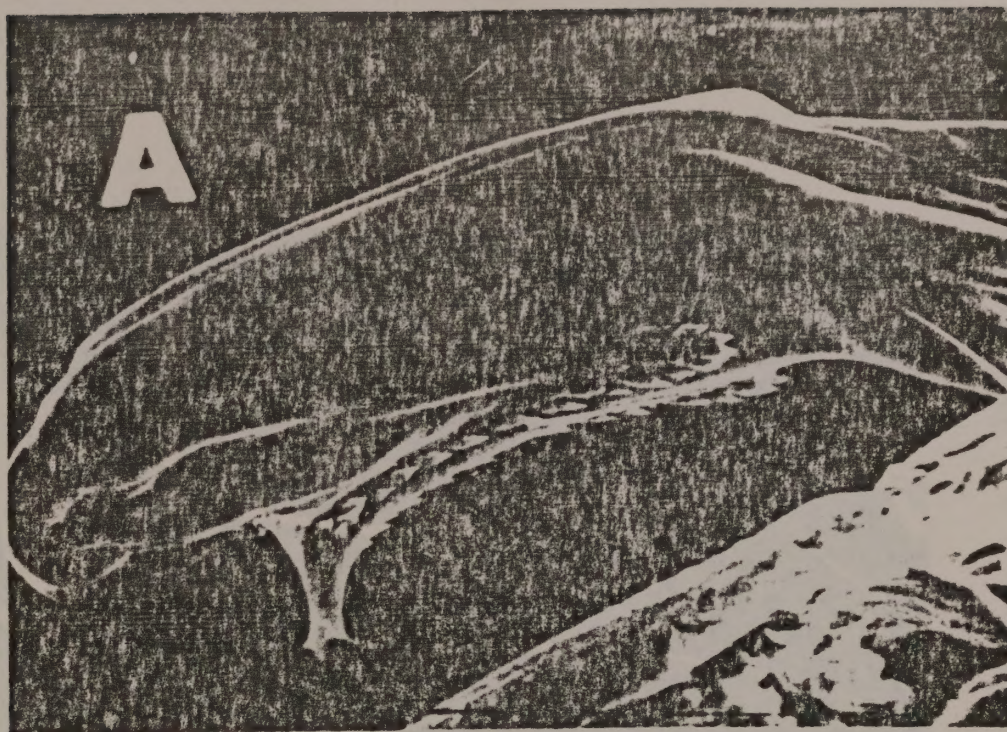


FIGURE 5: The tooth-like structures are in two rows on either side of the aedegus. The number of "teeth" is relatively constant within a sibship and probably within a type, but differs markedly with different types. Type A has characteristically 21 teeth, while type G has only 14. The orientation and the magnification of these micrographs are somewhat different. Type A is presented with a magnification of 290X while type G is magnified 410X.

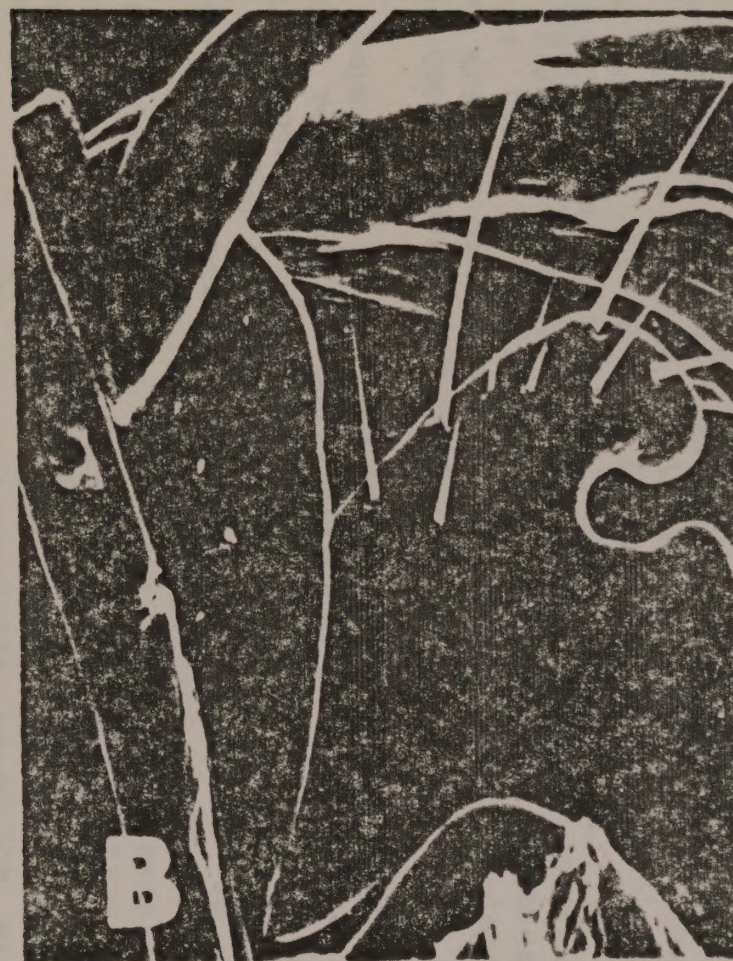


FIGURE 6: The gonopods vary among different types in bristle number, bristle placement, and in the overall shape and size of the structure itself. Types A and B differ in both bristle number and their placement. The entire ventral end of the Type A gonopod is free from bristles, while type B has two bristles in this region (only one is visible in this photograph).

bleached and stained. The genitalia are placed in a drop of water on a piece of double-stick tape on a mounting stob. The genitalia are then oriented using forceps. The water is frozen in liquid nitrogen and freeze-dried using a vacuum pump. The genitalia are then coated with platinum/gold using a sputter-coater (Varian Corp.).

Figure 4 shows some selected types of genitalia and enlargements of specific portions to show details of the differences between them. Of particular note are the vertical (Figure 4a) versus horizontal (Figure 4b) epithalis, the long wide paramere (Figure 4a), the short narrow paramere (Figure 4b) and the parameres that are held vertically (Figure 4g) versus those held horizontally (Figures 4a) or obliquely (Figure 4b). There are also differences in the hinging of the aedeagus with the ventral apodeme as seen in these photographs.

The comparative work on the morphology of the male genitalia is still in a relatively primitive state. Quantitative data for numerical methods of taxonomy can be obtained from the measurement of structures such as the number of tooth-like structures on the aedeagus (Figure 5), and noting the positions and number of the bristles on the gonopods (Figure 6). In these examples, significant differences can be seen between the types shown. From a comparatively small sample, characteristics such as the number of aedeagal teeth (14 in type A versus 21 in type G) and the number, position and size of gonopod bristles are reliable indicators of gamodeme type. There are undoubtedly others such indicators, either on the internal genitalia, or on the external genitalia.

The primary goal of this work is to develop a rapid field method of identifying the gamodeme type of a sample, and as a rapid check for contamination of cultures.

3.2 Problems With Sample Acquisition And Contamination

Gamodeme type is currently identified by observing chromosome type and electrophoretic patterns from late third instar larvae. When a sample is obtained by collecting a gravid female and rearing the offspring to late larval stages, then allowing them to pupate and rearing them to adults, then both larval and adult stages can be sampled. Twenty-two such sibships were obtained from the Arriaga region. These sibships showed clear differences in genitalia that were correlated with chromosome type. Egg-mass collections can be similarly reared and collected, but there is some possibility of collecting eggs from more than one gravid female. Of 8 samples examined from the collections (made by SEA) in Sinaloa, 2 showed heterogeneity in genitalia type (SS-51 and SS-54).

The problem of heterogeneity becomes much more significant when flies are cultured in the laboratories either at Mission or Tuxtla. To date, any stock more than 3 generations in culture has shown severe heterogeneity in genitalia morphology. The only reasonable explanation for such heterogeneity is contamination of the cultures.

The first step in the development of contamination-free culture procedures is to have a fast, accurate assay for strain purity. With further development, the morphology of the genitalia should provide such an assay. At present, the morphological differences described above can be used to detect most types of contamination.

4.0 COMMENTS ON GAMODEMES AND SPECIES

Much confusion has persisted over the genetic data showing mating structure in the natural populations, and in the relationship of this phenomenon to species. The details are academically important, and certain features are also of practical importance to this project, and to other programs for which this one serves as a model.

4.1 Origin Of "Gamodeme" Terminology

The term "gamodeme" was coined as part of a larger development of terminology. The people responsible were J. S. L. Gilmour, J. Heslop-Harrison and J. W. Gregor. The first attempt was in 1939, followed by the definitive paper in 1954 (Gilmour and Heslop-Harrison, "The deme terminology and the units of micro-evolutionary change." *Genetica* 27: 147-161). They defined a "gamodeme" as a group "composed of individuals which are so situated spatially and temporarily that, within the limits of the breeding system, all can interbreed." They allowed degrees of intermating, to be made explicit by whomever used the terms. "In framing the definitions of our terms, any implication of absolute standards of difference, interfertility, isolation and the like, has been rigidly excluded, and it is recognized that the categories give expression to findings which necessarily contain an element of the subjective." They devised this terminology to avoid the semantic arguments with taxonomists, where opinion reigned supreme, and usually objective testing was impractical or impossible. *tempo- rall*

4.2 Semantics And Concepts

The use of the term "species" is often made without a realization of the biological concepts needed for an unambiguous application of the term. Discussion of this term and the confusion associated with it, particularly by taxonomists of the "old school," forms a large part of G. G. Simpson's book, Principles of Animal Taxonomy (1961. Columbia University Press. New York. Chapters 4 and 5 are particularly pertinent.). Simpson defines a species as "... a lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies." This definition is an abstraction. It does not require morphological diversity nor any particular kind of impediments to gene flow (e.g. hybrid sterility). We must infer separate species when populations are evolving separately. Simpson, who is an eminent taxonomist, points out

that the term before Darwin's work referred to a very different concept than it does today. For most post-Darwinian taxonomists "... there was an ambiguous tendency to think of the species as an evolutionary unit but to try to define it typologically. Some taxonomists still do not understand or do not accept the distinction, and the continuing ambiguity is one of the reasons for the 'species problem' -- the problem, which has probably caused more ink to flow than any other one point in taxonomy, is to produce a generally acceptable and workable definition of the category called species."

4.3 Cutting Through The Semantics

To us, the major problem is the distraction from relating the gamodemes to the screwworm program when "species" is mentioned. It actually is not important operationally what term we use, of course, and the distractions come from the uncertainty of how much or how little mating will occur under the conditions of the sterile fly releases on different gamodemes. There are other related problems as well. These are centered on the organization of the genetic systems that have evolved in the different gamodemes. And, while we have not discussed it very much, there are problems concerning the plasticity of natural populations to again produce economic problems after the program has been declared a success. And, finally, it is important to understand why this program succeeds or why difficulties may arise, since it will continue to serve as a model for pest control using autocidal approaches (E. F. Knipling and W. Klassen, personal communication).

4.4 Tests And Techniques

Rather than concentrate limited resources on tests conducted under conditions very different from either natural populations of screwworms or conditions of the eradication program, techniques simulating more relevant conditions are badly needed. Field tests are complex, very expensive, and many times impossible to conduct to give unambiguous results. A much simpler and less expensive procedure is needed which can be calibrated to results in the field. This is the idea behind Wil Averhoff's work in the Green Room at Tuxtla Gutierrez.

We Need to Know ...

To our knowledge, there are no laboratory or field tests of screwworms that unequivocally, or even very probably, were conducted with pure strains, or known strains. Possibly the "Florida Strain" while used in Florida comes the closest. A critical re-evaluation of all experiments and field results is badly needed to allow

results to be declared ambiguous, or to allow any results without a cloud of doubt to be highlighted. For ambiguous results, additional explanations may be possible in light of our increasing knowledge of genetic diversity.

4.5 Assumptions, Axioms And Generalizations

Tests to determine the consequences of genetic divergence among gamodememes for use in the factory could become of academic interest only. The question could be avoided for practical purposes. If the gamodememes were not intercrossed, disruption of their genetic systems would not be possible. A discussion detailing the genetic principles and experimental justification will be prepared in the near future. For the moment, however, it is maybe appropriate to repeat the often heard observations and expressed objectives to produce an effective factory population in conjunction with the things we know from basic principles of population genetics.

1. The best population is one that remains as similar to the wild population ("target population") as possible.
2. The artificial culture, whether in SEA, Methods Development or the factory, imposes strong artificial selection pressures on the population. It is axiomatic that this selection will cause the population to be less like the target population. The divergence ultimately will be limited only by the exhaustion of genetic variation in the factory population. (Fisher, 1918; Bell, Moore and Warren, 1955; Clayton and Robertson, 1957; Tantawy and Reeve, 1956), except for that genetic variation resulting from complex interactions and linkages (Kojima and Kelleher, 1963; Richardson and Kojima, 1965).
3. The objective of combining several samples, whether from a single locality or from widely different areas of the intended drop zone, is to obtain a sample representative of the target population. A few years ago a correct answer was given for the wrong question. ("How many eggmasses should be collected to be assured of having a representative of most of the genetic diversity in the wild population?") The result of this question has been a persistent adherence to forming a factory population from an intercrossing among descendants of 30-50 parents (15-25 egg masses), plus an unknown quantity of individuals of unknown genetic constitution from the contaminants of the lines. The end product that is dropped on the target population is the important population, as all would recognize, and the sampling is only a part of the picture. The question NOT asked is how to keep the factory population like the sample! In our opinion the sample size is much less important than what population is

*Not supported by the
results of Sinaloa & Vera*

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sampld and what happens to the sample before it produces sterile flies.

4. Regardless of whether we refer to the genetic diversity we have found as sub-specific or specific, it is genetic diversity. Thus, we are not dealing with THE target population, but with several populations. In fact, the recognition of genetic diversity among screwworms was evidenced as soon as sampling for the factory population was done from different areas. If one wishes to obtain factory populations similar to the native populations, an admixture of gene pools is different from either -- wine and milk mixed together do not give a reasonable approximation of either original component. Raisins and peanuts mixed together may not be so far away, and one who prefers peanuts may exclude the raisins, even in the dark. The possibilities of hybrid breakdown are extremely high for a population which is freely recombining differentiated gene pools (McInnis reviewed part of the relevant literature in his memorandum to Leo LaChance) and the degree of detrimental effects may be much more extreme where the divergence is at or near that differentiating species.

At the very least, the genetic variation is increased, and the effectiveness of artificial selection under culture conditions is greatly enhanced. There is a real hazard in following this procedure. Such a population was recently produced by Bruce Wallace and a student (Cornell University, personal communication) in which individuals discriminated against both parental populations.

5. Continued inbreeding does, indeed, produce homozygosity up to the point an equilibrium is reached with selection for heterotic genes or linked blocks of genes. The loss of genetic variation followed by bottlenecking a population for one generation, followed by rapid expansion (i.e. each parent has many offspring) is essentially determined by the heterozygosity of the individuals at the time of the bottleneck. If, say, a single pair of parents represented the bottleneck, and each were heterozygous for all loci at which their population was segregating, then the only loss in genetic variation would be at loci for which there were more than 4 alleles. (Each member of this pair was formed by two gametes, and could carry two different alleles.) Of course, all loci would not be expected to be heterozygous, but if the two individuals were taken from an outbred population, a large part of the genome in each of these two individuals would be heterozygous. Furthermore, if they were unrelated, they likely would be heterozygous for many different loci. In studies of evolutionary biology the production of a new population via a single pair followed by rapid expansion is known as a "founder event" and has been studied extensively by many geneticists (reviewed by Nei, 1975; Templeton and Rankin, 1978; Templeton, 1979). The adverse effects of inbreeding generally are absent, yet the bottleneck partially limits the genetic variation in the new population,

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and greatly limits the release of the variation which requires genetic recombination. (From our samples of factory populations this type of population is present as a spectrum of contaminants which presumably do not freely interbreed with the population originally concocted. The present DE-9 factory population has a high proportion of males with genitalia characteristic of "West Coast" screwworms, whereas the original population was exclusively from the east coast.)

6. Tests of components of fitness that are important to mating of sterile flies with fertile flies include only part of the total fitness acted on in nature by natural selection. It is a moot point in most cases what the effects of artificial selection will be on these important fitness components for effective suppression of the target population by the sterile released population. It is very difficult to use data from tests conducted under artificial conditions to predict the effectiveness of released populations under natural conditions. Therefore, the rapid replacement of a factory population when it becomes ineffective constitutes a flexible and almost certainly successful strategy. Any procedure that prolongs the time from collection of native germplasm to its release as a sterile population will reduce the chances for the most efficient population suppression. Furthermore, it has been repeatedly suggested on firm genetic principles that the factory population should be genetically similar to the target population (Beal, 1974; Richardson, Ellison and Averhoff, 1978; many others). Samples taken from the cases that occur during the release of sterile flies represent those females not sterilized. Factory populations derived from such "escapes" should have a good chance in being effective against the descendants of the escapes. Of course, where more detailed information is available, further improvement in the chances of producing effective sterile flies may be achieved. Obtaining this information is the objective in this study.

5.0 TUXTLA STUDIES

5.1 A Study Of Mate Selection In Screwworm Flies

Continuous production of high quality, effective screw-worm males for sterile male release requires an understanding and convenient means of testing both mating behavior and mate selection under natural conditions or (as a first approximation) under conditions in which screw-worms behave normally. Crucial aspects of mate selection remain unknown.

Recently, observations of adult factory screw-worms around flowering plants (Mackley) have provided the program with descriptions of mating behavior that offer considerable insight into devising experimentally tractable procedures for testing features of mating behavior and mate discrimination. While Mackley's observations represent

artificial situations of high density of factory-reared flies in a context that is not flexible experimentally, his observations suggest many variables to examine in devising a scheme for experimentally testing. Hopefully, results may be extrapolated to field conditions. Other studies using flies physically restrained in cages have not successfully reproduced a full mating sequence as described from field observations of released flies. Caged flies usually go into escape behavior and males do not orient and chase females (Graham). Though mating between genetic types occurs in cages, crucial behavioral sequences of both males and females are short circuited producing crosses that do not occur in nature. Even so, some genetic isolation has been reported in lab cage populations (McInnis).

An alternative approach, already in progress at Tuxtla has been outlined and discussed. This approach, exploiting the natural behavioral responses of screwworms toward temperature, humidity and light gradients, is now being pursued in Tuxtla G. The approach, though straightforward biologically, requires fairly complex physical equipment and manipulations to generate appropriate gradients of these physical parameters compatible with screw-worm preferences. Within these limits, manipulations of numerous biological parameters can be obtained. Flies behaviorally confined will, it is hoped, respond more naturally in mating situations.

5.2 Facilities And Design

The initial space allotment for this work was volunteered by Dr. Meadows of APHIS in March 1979. The "Green Room" of Methods and Development at the Tuxtla plant was chosen with the help and advice of the people there. After initial observations in the room in late 1979, Dr. Wil Averhoff requested more refrigeration capacity, which has recently been added to room and tested. However, as of this moment it is being used to cool all rooms of Methods Development until repairs can be completed in the normal refrigeration for the area.

A black-flannel room-liner is currently in place as a barrier to contain flies and avoid outside disturbances but also to direct and modify cold air flow and absorb light reflections. Heating pads, coils and humidifying systems are now in place. The controlled space is of sufficient size to accommodate live animals for oviposition sites.

5.3 Research Plans

Averhoff took cultures from Mission to Tuxtla G. Cultures include those from Arriaga as well as other collections from elsewhere in Mexico. Behavioral restraint tests in the green room will begin as soon as possible, when the temperature gradient once again can be manipulated. Flies' reactions to various gradients will be continuously monitored until behaviorally delimited gradients in temperature, humidity and light, suitable to contain screw-worms have been

determined. If flies positively confined (that is, remain within preferred portion of gradients) behave as expected, tests will then focus on isolation and mate selection. The ultimate goal will be to determine strains most suitable (i.e. express the fewest mating barriers between the greatest number of types) for release in a given area.

5.4 Field Studies (Chiapas, Mexico)

1. July - August 1979 -- Survey collections of Arriaga area are the bases for much of genetic analysis above.
2. September - October 1979 -- Collections of cultures for field tests from cows and sentinel sheep were made in area from Arriaga to Pijijiapan. Isofemale lines started at this time in Tuxtla were later lost and/or contaminated.
3. March - May 1980 -- New isofemale lines were begun from material collected from sentinel sheep. Isofemale lines were reared to pupation in Tuxtla then moved and maintained in Mission. At this time field conditions were extremely dry limiting collections from sheep and no cases could be obtained from livestock populations along coast. One case, however, was collected from a calf in mountains above Arriaga.

Recollection of this area provided valuable information on seasonal fluctuations of population density. Not only does density in this area change but the proportion of types appears to change as well. Collections were limited because of seasonal drought, hence more accurate assessment of seasonal changes in this area should be determined.

5.5 Behavior

The unobtrusive oviposition behavior of female screwworms around wounded animals contrasts sharply with that of other blowflies. The screwworm female arrives at wounds silently without persistent buzzing of wounded area and simply moves away from wound if the animal is annoyed and shakes, licks, or rubs the wound. Again this contrasts with behavior of other blowflies which buzz the wound landing on ears, face and nose of animal. Female screwworms ready to oviposit generally spends no more than a few minutes in or on wound before oviposition. Though she may feed briefly, screwworms generally leave wound shortly after oviposition.

5.6 Blowflies

Another approach to the study of screwworm mating behavior is to study the behavior of closely related but more easily observed blowflies and apply knowledge and techniques learned to screwworms. Field observations and laboratory experiments are now in progress on the screwworm congener, C. macellaria.

This blowfly, though differing in larval substrate and behavior around wounded animals, appears to have largely similar mating and courtship habits. This is also one of the most common flies in the southwestern U.S., making observations in the field much easier than those on the screwworm. Field and laboratory experiments are currently in progress at the University of Texas, Brackenridge Field Laboratory in Austin.

Laboratory experiments on contact exoskeleton pheromones are in progress. Preliminary observations indicate males respond to non-volatile exoskeleton bound pheromones carried in exoskeleton of virgin females. Mating response seems tied to these contact signals. In conjunction with laboratory experiments rearing and handling techniques are being developed and tested on other blowflies. Extension of results on C. macellaria to other blowflies are planned so that these in turn may be used to develop testable hypotheses about evolutionary trends in the group especially as they give clues to behavior of screwworms. An independent measure of evolutionary relatedness within the tribe based on molecular characterization is planned in conjunction with this work.

